

U.S.S.N. 09/494,585
Filed: January 31, 2000

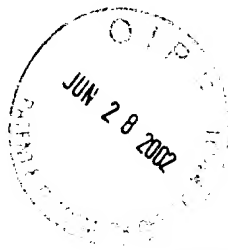


Exhibit 1

Declaration of William LaRochelle under 37 C.F.R. §1.132

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PATENT APPLICATION
ATTY DOCKET 15966-557 (CURA-57)



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS : Shimkets *et al.*
SERIAL NUMBER : 09/494,585
FILING DATE : January 31, 2000
FOR : NOVEL FIBROBLAST GROWTH FACTOR AND NUCLEIC ACIDS ENCODING SAME

EXAMINER : Christine Saoud
ART UNIT : 1647

#74
1/11/2

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, William LaRochelle, of Madison, CT, hereby declare and state as follows:

1. I am employed by CuraGen, Inc., the assignee of this application. My title is Group Leader. I received a Ph.D. in 1987 from Dartmouth Medical School, Department of Biochemistry. I was also an American Cancer Society postdoctoral fellow at the National Cancer Institute (1987-1990). From 1990-2000, I was a Senior Staff Fellow/Principal Investigator at the Laboratory of Cellular and Molecular Biology at the National Cancer Institute, Bethesda, MD.
2. I have read the Office Action mailed on December 28, 2001 and am familiar with the Examiner's grounds of rejection of the pending claims.
3. The oligonucleotide primers pSec-V5-His Forward (CTCGT CCTCG AGGGT AAGCC TATCC CTAAC (SEQ ID NO:14)) and pSec-V5-His Reverse (CTCGT CGGGC CCCTG ATCAG CGGGT TTAAA C (SEQ ID NO:15)), were designed to amplify a fragment from the pcDNA3.1-V5His (Invitrogen, Carlsbad, CA) expression vector that includes V5 and His6. The PCR product was digested with XhoI and Apal and ligated into the XhoI/Apal digested pSecTag2 B vector harboring an Ig kappa leader sequence (Invitrogen, Carlsbad CA). The correct structure of the resulting vector, pSecV5His, including an in-frame Ig-kappa leader and V5-His6 was verified by DNA sequence analysis. The vector pSecV5His was digested with PmeI and NheI to provide a fragment retaining the above elements in the correct frame. The PmeI-NheI

fragment was ligated into the BamHI/Klenow and NheI treated vector pCEP4 (Invitrogen, Carlsbad, CA). The resulting vector was named pCEP4/Sec and includes an in-frame Ig kappa leader, a site for insertion of a clone of interest, and the V5 epitope and 6xHis under control of the PCMV and/or the PT7 promoter. pCEP4/Sec is an expression vector that allows heterologous protein expression and secretion by fusing any protein into a multiple cloning site following the Ig kappa chain signal peptide. Detection and purification of the expressed protein are aided by the presence of the V5 epitope tag and 6xHis tag at the C-terminus (Invitrogen, Carlsbad, CA).

4. The expression of FGF-CX in human embryonic kidney (HEK) 293 cells, the BglII-XhoI fragment containing the FGF-CX sequence was isolated from TA- AB02085-S274-F19 (as set forth in specification of the instant application as Example 1) and subcloned into the BamHI-XhoI digested pCEP4/Sec to generate the expression vector pCEP4/Sec-FGF-CX. The pCEP4/Sec-FGF-CX vector was transfected into 293 cells using the LipofectaminePlus reagent following the manufacturer's instructions (Gibco/BRL/Life Technologies, Rockville, MD). The cell pellet and supernatant were harvested 72 hours after transfection and examined for FGF-CX expression by Western blotting (reducing conditions) with an anti-V5 antibody. Fig. 12 shows that FGF-CX is expressed as a polypeptide having an apparent molecular weight (Mr) of approximately 34 kDa proteins secreted by 293 cells. In addition a minor band is observed at about 31 kDa.

5. Stimulation of bromodeoxyuridine incorporation by recombinant FGF-CX was performed using the following protocol. 293-EBNA cells (Invitrogen) were transfected with pCEP4/Sec-FGF-CX vector using Lipofectamine 2000 according to the manufacturer's protocol (Life Technologies, Gaithersburg, MD). Cells were supplemented with 10% fetal bovine serum (FBS; Life Technologies) 5 hr post-transfection. To generate protein for BrdU and growth assays, cells were washed and fed with Dulbecco's modified Eagle medium (DMEM; Life Technologies) 18

hr post-transfection. After 48 hr, the media was discarded and the cell monolayer was incubated with 100 μ M suramin (Sigma, St. Louis, MO) in 0.5 ml DMEM for 30 min at 4°C. The suramin-extracted conditioned media was then removed, clarified by centrifugation (5 min; 2000 X g), and subjected to TALON metal affinity chromatography according to the manufacturer's instructions (Clontech, Palo Alto, CA) taking advantage of the carboxy-terminal polyhistidine tag. Retained fusion protein was released by washing the column with imidazole.

FGF-CX protein concentrations were estimated by Western analysis using a standard curve generated with a V5-tagged protein of known concentration. For Western analysis, conditioned media was harvested 48 hr post transfection, and the cell monolayer was then incubated with 0.5 ml DMEM containing 100 μ M suramin for 30 min at 4°C. The suramin-containing conditioned media was then harvested.

To generate control protein, 293-EBNA cells were transfected with pCEP4 plasmid (Invitrogen) and subjected to the purification procedure outlined above.

Recombinant FGF-CX was tested for its ability to induce DNA synthesis in a bromodeoxyuridine (BrdU) incorporation assay. NIH 3T3 cells (ATCC number CRL-1658, American Type Culture Collection, Manassas, VA), CCD-1070Sk cells (ATCC Number CRL-2091) or MG-63 cells (ATCC Number CRL-1427) were cultured in 96-well plates to ~100% confluence, washed with DMEM, and serum-starved in DMEM for 24 hr (NIH 3T3) or 48 hr (CCD-1070Sk and MG-63). Recombinant FGF-CX or control protein was then added to the cells for 18 hr. The BrdU assay was performed according to the manufacturer's specifications (Roche Molecular Biochemicals, Indianapolis, IN) using a 5 hr BrdU incorporation time.

It was found that FGF-CX induced DNA synthesis in NIH 3T3 mouse fibroblasts at a half maximal concentration of ~5 ng/ml (Figure 1, Panel A). In contrast, protein purified from cells transfected with control vector did not induce DNA synthesis. It was also found that FGF-CX induces DNA synthesis, as determined by BrdU incorporation, at comparable dosing levels in a variety of human cell lines including CCD-1070Sk normal human skin fibroblasts (Figure 1,

Panel B), CCD-1106 keratinocytes (Figure 1, Panel C), MG-63 osteosarcoma cells (data not shown), and breast epithelial cells.

Figure 1.

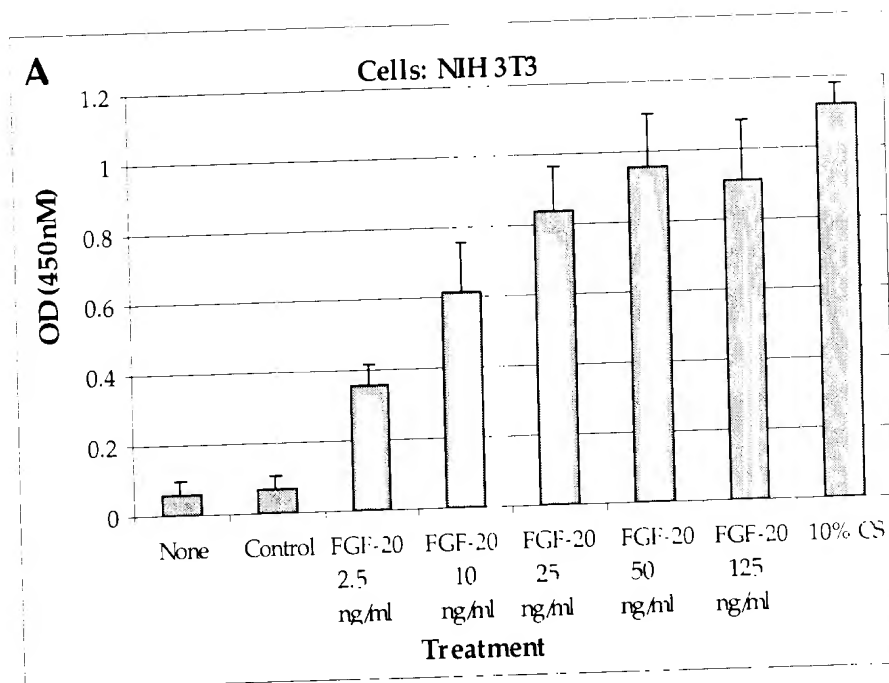
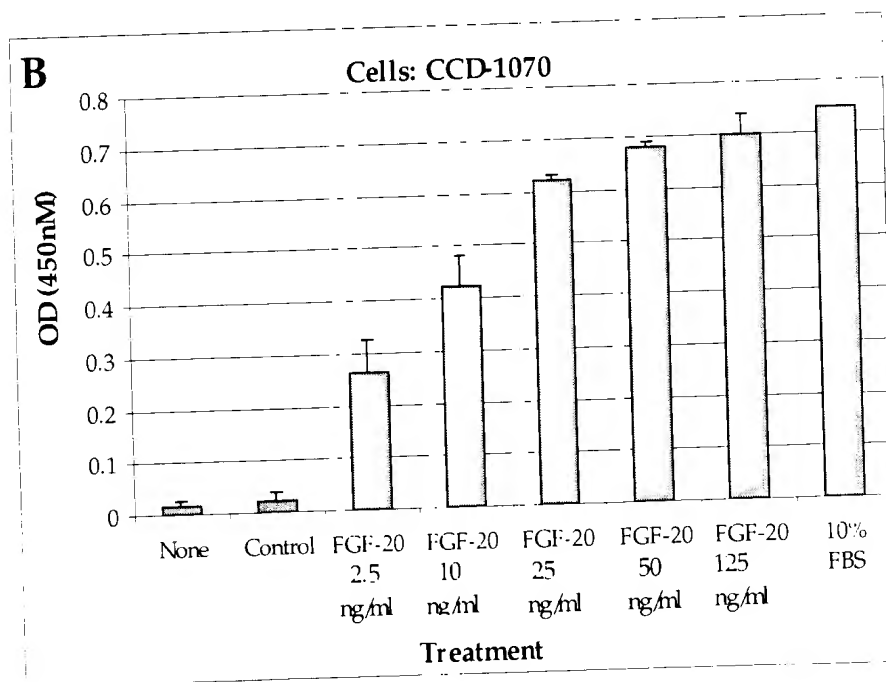
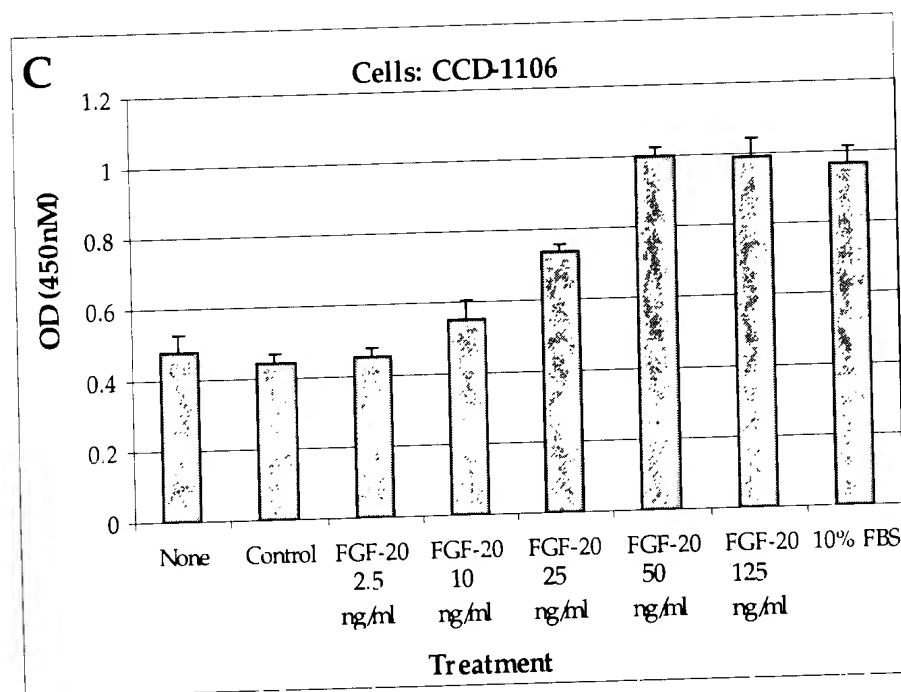


Figure 1 (continued)





6. To determine if recombinant FGF-CX induces cell proliferation, NIH 3T3 cells were cultured in 6-well plates to ~50% confluence, washed with DMEM, and fed with DMEM containing recombinant FGF-CX or control protein for 48 hr, and then counted. Cell numbers were determined by trypsinizing the cells and counting them with a Beckman Coulter Z1 series counter (Beckman Coulter, Fullerton, CA). It was found that FGF-CX induces about a 3-fold increase in cell number relative to control protein in this assay (Figure 2).

To document morphological changes incident upon proliferation, NIH 3T3 cells were treated for 48 hr with recombinant FGF-CX or control protein in DMEM/2% calf serum and

photographed with a Zeiss Axiovert 100 microscope (Carl Zeiss, Inc., Thornwood, NY), the results of which are shown in Figure 3.

These results show that FGF-CX acts as a growth factor and suggest that recombinant FGF-CX mediates the morphological transformation of NIH 3T3 cells.

Figure 2.

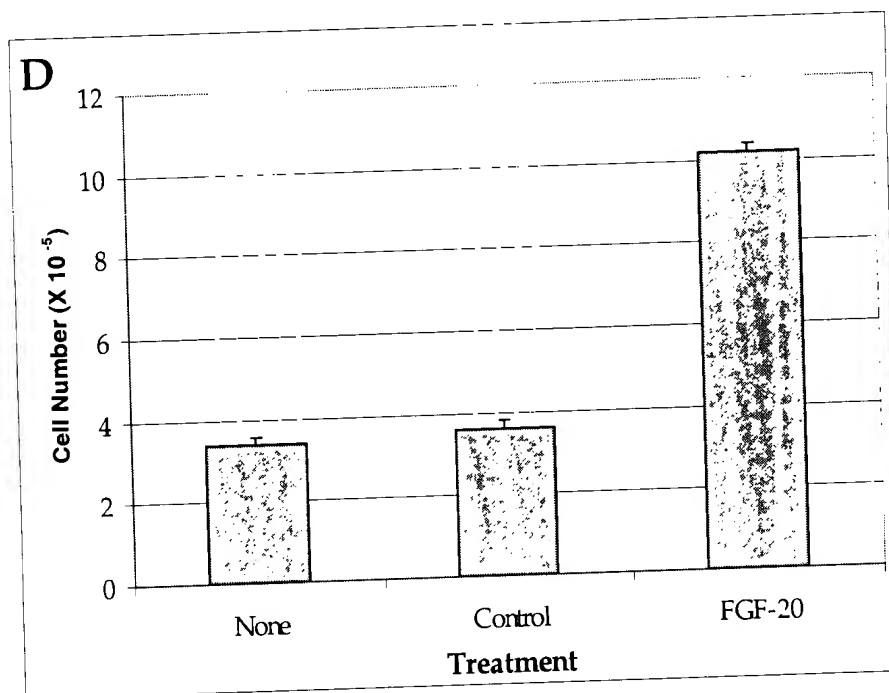
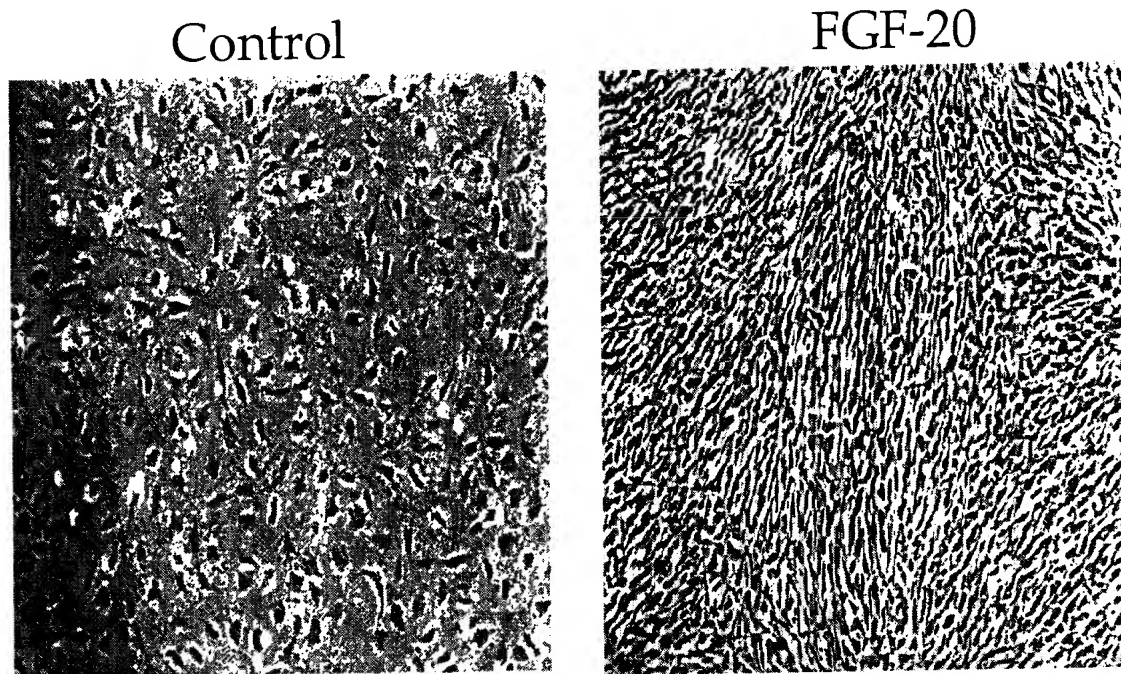


Figure 3.



7. It is my firm belief, based on the literature and my own experience, that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by a fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

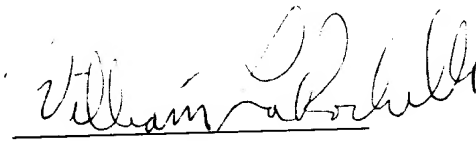
8. For all the foregoing reasons, I believe that the Examiner should withdraw the rejection and allow the pending claims.

Inventors: Shimkets and Prayaga
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9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Date:

6-19-00


William LaRochelle

TRA 1680378v1